

AMENDMENTS TO THE CLAIMS

~~We Claim~~ What is claimed is:

Claim 1 (Original): A novel protein capable of inhibiting anthrax toxin activity said protein comprising of following characteristics:

- (i) Hydrophobic in nature,
- (ii) Molecular weight 67 kDa,
- (iii) Stable at room temperature,
- (iv) Resistant to trypsin,
- (v) Having no proteolytic activity,
- (vi) Inhibits proteolytic cleavage of protective antigen (PA) of B. anthracis in a dose dependent manner,
- (vii) Binds to IgE, and
- (viii) The protein is devoid of any carbohydrate moiety.

Claim 2 (Currently Amended): ~~A~~The protein ~~as claimed~~of claim 1 wherein the ~~said~~ protein is isolated from the pollen grains of grass species selected from group of Imperata cylindrica (Ic), Lolium perenne, Phleum pratense, Cynodon dactylon and related genus.

Claim 3 (Currently Amended): ~~A~~The protein ~~as claimed in~~of claim 1 wherein the said protein is stable in the temperature range of about 3°C to 40°C

Claim 4 (Currently Amended): ~~A~~The protein ~~as claimed in~~of claim 3 wherein ~~the said~~ the protein is stable in the temperature range of about 4°C to 37°C.

Claim 5 (Currently Amended): ~~A~~The protein ~~as claimed in~~of claim 1, wherein protein in the range of about 25-20 ng completely inhibits the protective antigen (PA) of the anthrax toxin.

Claim 6 (Currently Amended): ~~A~~The protein ~~as claimed in~~of claim 1, wherein the protein in the range of about 15-5 ng partially blocks the cleavage activity of the PA.

Claim 7 (Currently Amended): ~~A~~The protein ~~as claimed~~of claim 1, wherein the protein in the range of about 25 ng to 11,000 ng is ~~efficient~~ effective in inhibiting the anthrax toxin activity.

Claim 8 (Currently Amended): ~~A~~The protein ~~as claimed in~~claim 1, wherein the protein in the range of about 50 ng to 10,000 ng is ~~efficient~~ effective in inhibiting the anthrax toxin activity.

Claim 9 (Original): A process of isolating the novel protein capable of inhibiting anthrax toxin activity, said process comprising steps of:

- (i) extracting the total protein from the grass pollen by suspending the pollen in phosphate buffer for a period of about 3h to 15 h under stirring continuously under cold conditions followed by high speed centrifugation at 15,000 rpm,
- (ii) purifying protein fractions from the extract of step (i) by column chromatography,
- (iii) lyophilizing the dialyzed protein fraction containing the protein of interest obtained in step (ii).
- (iv) subjecting the protein fractions of step (iv) to SDS-PAGE followed by Western blotting and immuno-staining to separate and locate the protein of interest,
- (v) testing the ability of the purified protein to inhibit anthrax toxin activity by incubating the isolated protective antigen (PA) of B. anthracis with or without lyophilized isolated protein from a grass in presence of trypsin for measuring the PA cleaving (inhibitory) activity of the isolated protein by SDS-PAGE in a dose dependent manner.
- (vi) characterizing the purified protein allergenic activity by SDS-PAGE, Western blotting and immuno-staining.

Claim 10 (Currently Amended): ~~A~~The process ~~as claimed in claims 13~~of claim 9, wherein the pollen grains for purification of the protein in the step (i) are collected from grasses selected from group comprising of Imperata cylindrica (Ic), Lolium perenne, Phleum pratense, Cynodon dactylon and related genus.

Claim 11 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9 wherein the buffer used for extraction of pollen in the step (i) is selected from group comprising of 0.1M PBS or 0.1 M ammonium bicarbonate of pH ranging from 7.0 to 8.0.

Claim 12 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9 wherein the material used for the column chromatography in step (ii) is a hydrophobic resin selected from octadecyl silica gel and similar silica gels.

Claim 13 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9, wherein the protein bound to the chromatography column in step (iii) is eluted with acetonitrile in range of about 30-75% and about 0.50 z% Trifluoroacetic acid (TFA) in water.

Claim 14 (Currently Amended): ~~A process as claimed in claim 17~~The process of claim 9, wherein the acetonitrile is in the range of about 40-60% and TFA is about 0.1% in water.

Claim 15 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9, wherein the protein obtained in step (vi) is stable in the temperature range of about 3°C to 40°C

Claim 16 (Currently Amended): ~~A process as claimed protein as claimed in claim 19~~The process of claim 9 wherein the said the protein is stable in the temperature range of about 4°C to 37°C.

Claim 17 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9, wherein the protein obtained in the range of about 25-20 ng completely inhibits the protective antigen (PA) of the anthrax toxin.

Claim 18 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9, wherein the protein obtained in the range of about 15-5 ng partially blocks the cleavage activity of the PA.

Claim 19 (Currently Amended): ~~A process as claimed in claim 13~~The process of claim 9, wherein the protein obtained in the range of about 25 ng to 11,000 ng is ~~efficient~~effective in inhibiting the anthrax toxin activity.

Claim 20 (Currently Amended): ~~A process as claimed in claim 25~~The
process of claim 9, wherein the protein obtained in the range of
about 50 ng to 10,000 ng is ~~efficient~~effective in inhibiting the
anthrax toxin activity.